AD_____

(Supersedes ADA213392)

GRANT NO: DAMD17-89-Z-9021

TITLE: DISCOVERY AND DEVELOPMENT OF THERAPEUTIC DRUGS AGAINST LETHAL HUMAN RNA-VIRUSES: A MULTIDISCIPLINARY ASSAULT

PRINCIPAL INVESTIGATOR: Dr. George R. Pettit

CONTRACTING ORGANIZATION: Cancer Research Institute
Arizona State University
Tempe, Arizona 85287-2404

REPORT DATE: February 20, 1990

TYPE OF REPORT: Midterm Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Cancer Research Institute; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Arizona State University, Tempe, Arizona	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-89-Z-9021
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62787A	3M1-62787A871	AB	374

11. TITLE (Include Security Classification)
DISCOVERY AND DEVELOPMENT OF THERAPEUTIC DRUGS AGAINST LETHAL HUMAN RNA-VIRUSES: A MULTIDISCIPLINARY ASSAULT

12. PERSONAL AUTHOR(S)
George R. Pettit

13a. TYPE OF REPORT	13b. TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Midterm Report	FROM 2/6/89 TO 2/5/90	1990 February 20	25

16. SUPPLEMENTARY NOTATION
Supersedes DTIC ADA213392.

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	RA I; Antivirals; Natural products; RNA Viruses; BD; Discovery of RNA-type antiviral drugs, naturally occurring antiviral drugs
06	03		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The grant funds have been sharply focused on discovery of new antiviral drugs. We have been pursuing the pancratistatin family of antiviral leads as a top priority. The research results here have been very encouraging and pancratistatin, isonarciclasine, cis-dihydronarciclasine as well as trans-dihydronarciclasine have proved to be quite promising. Meanwhile over 1700 naturally occurring specimens are now undergoing preliminary antiviral evaluation at USAMRIID. The resulting leads will also be pursued as rapidly as financial resources permit. The most exciting overall result has been the discovery in USAMRIID's laboratories that pancratistatin will cure the in vivo experimental version of Japanese Encephalitis. In addition, of the 1770 samples submitted during this period for pre-screen testing, we have received data for 875. Of these 150 samples displayed possible activity against either PT or YF or both for a total of 173 positive results.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mrs. Virginia M. Miller	301/663-7325	SGRD-RMI-S

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

X Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

X Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Accession For	
NTIS CRA&I	X
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

PI - Signature

3/20/90
Date

TABLE OF CONTENTS

INTRODUCTION

To summarize, a long-term USAMRIID research program directed at the isolation and structural elucidation of new and potentially useful antiviral drugs from marine animals and plants is in progress. The financial support provided by the USAMRIID program will continue to be used to isolate and characterize new antiviral chemotherapeutic drugs from confirmed active extracts of marine invertebrates and vertebrates as well as marine and terrestrial plants including fungi, algae and other microorganisms. The research is sharply directed at marine animal and plant species yielding extracts with an outstanding level of antiviral activity in the USAMRIID's programs (RNA viruses).

BODY

The overall results and status to date are as follows. The grant funds are being used for discovery of new antiviral drugs. We have been pursuing the pancratistatin family of antiviral leads as a top priority. The research results here have been very encouraging and pancratistatin, isonarciclasine, cis-dihydronarciclasine as well as trans-dihydronarciclasine have proved to be quite promising. Meanwhile over 1700 naturally occurring specimens are now undergoing preliminary antiviral evaluation at USAMRIID. The most exciting overall result has been the discovery in USAMRIID's laboratories that pancratistatin will cure the in vivo experimental version of Japanese Encephalitis. In addition, of the 1770 samples submitted during this period for pre-screen testing, we have received data for 875. Of these 150 samples displayed possible activity against either PT or YF or both for a total of 173 positive results.

To follow is a detailed report of the latest antiviral evaluation results as Part A. In part B appears experimental results corresponding to our synthetic transformations aimed at uncovering important antiviral derivatives of pancratistatin and developing a workable synthetic approach to pancratistatin based on narciclasine. Meanwhile we have completed reisolation of pancratistatin from 1/2 ton of Pancratium littorale collected in the Republic of Seychelles. Enough pancratistatin is now available for the next series of in vivo experiments. In short progress continues to be excellent and we have a magnificent number of new antiviral leads to pursue.

Also an outline summary of fractions obtained from the Papua New Guinea sponge B723344 and the Philippines marine sponge B721160 is attached.

A major effort has been devoted to scaling-up the isolation of narciclasine from a Narcissus species. We will shortly have 10 grams of narciclasine ready for chemical conversions and we will be concentrating on increasingly larger scale-up operations using the Narcissus with the objective of obtaining about 100+ grams of narciclasine.

In summary we are making excellent progress and this has been made possible by the most necessary USAMRIID support.

USAMRIID ACTIVES (Contract)

ASU NUM	AREA	TYPE	VIRUS	DATA SCREEN	DATE RECD	DRUG NUM	AVS NUM
B23-11C			YF	22.03 Pre	11/14/89		4855
B23-11C			PT	12.27 Pre	11/14/89		4855
M-2927	Philippines.79	Echinodermata	PT	12.35 Pre	09/28/89	B721092	1312
M-2928	Philippines.79	Echinodermata	PT	2.21 Pre	09/28/89	B721095	1315
M-3070	Philippines.79	Porifera	PT	2.23 Pre	11/14/89	B721511	1801
M-3077	Philippines.79	Porifera	PT	1.19 Pre	09/28/89	B721532	1818
M-3093	Philippines.79	Porifera	PT	13.49 Pre	09/28/89	B721568	1850
M-3093	Philippines.79	Porifera	PT	34.08 Pre	11/14/89	"	1850
M-3093	Philippines.79	Porifera	YF	22.24 Pre	09/28/89	"	1850
M-3312	Australia.78	Coelenterata	PT	1.90 Pre	11/14/89	B721743	
M-3314	Australia.78	Coelenterata	PT	2.18 Pre	11/14/89	B721749	6584
M-3328	Australia.78	Chordata/Tunicata	PT	1.67 Pre	10/31/89	B721787	6585
M-3136	Palau.79	Mollusca	PT	1.33 Pre	10/31/89	B721818	6586
M-3137	Palau.79	Porifera	PT	5.16 Pre	10/31/89	B721823	6248
M-3137	Palau.79	Porifera	PT	7.29 Pre	11/20/89	"	6248
M-3137	Palau.79	Porifera	YF	6.51 Pre	10/31/89	"	6248
M-3137	Palau.79	Porifera	YF	1.74 Pre	01/02/90	"	6248
M-3138	Palau.79	Porifera	PT	1.82 Pre	10/31/89	B721826	6587
M-3142	Palau.79	Echinodermata	PT	3.73 Pre	10/31/89	B721838	6588
M-3156	Palau.79	Porifera	PT	13.99 Pre	10/31/89	B721880	6589
M-3160	Palau.79	Chordata/Pisces	PT	1.02 Pre	10/31/89	B721892	6590
M-3163	Palau.79	Chordata/Pisces	YF	10.52 Pre	01/02/90	B721899	6591
M-3163	Palau.79	Chordata/Pisces	PT	5.84 Pre	01/02/90	"	6591
M-3165	Palau.79	Chordata/Pisces	PT	5.56 Pre	01/02/90	B721905	6592
M-3166	Palau.79	Chordata/Pisces	PT	3.29 Pre	11/09/89	B721908	6593
M-3166	Palau.79	Chordata/Pisces	PT	3.56 Pre	11/09/89	B721910	6594
M-3169	Palau.79	Chordata/Pisces	PT	7.41 Pre	11/09/89	B721917	6595
M-3171	Palau.79	Chordata/Pisces	PT	2.16 Pre	11/09/89	B721925	6596
M-3181	Palau.79	Chordata/Pisces	PT	2.69 Pre	01/02/90	B721953	6597
M-3181	Palau.79	Chordata/Pisces	YF	3.13 Pre	01/02/90	"	6597
M-3182	Palau.79	Chordata/Pisces	PT	1.63 Pre	11/20/89	B721958	6598
M-3189	Palau.79	Chordata/Pisces	PT	3.35 Pre	01/02/90	B721979	6599
M-3198	Palau.79	Chordata/Pisces	PT	1.32 Pre	11/20/89	B722006	6600
M-3212	Palau.79	Porifera	PT	8.13 Pre	01/02/90	B722048	6601
M-3214	Palau.79	Porifera	PT	3.10 Pre	01/02/90	B722052	6602
M-3214	Palau.79	Porifera	PT	27.94 Pre	01/09/90	B722054	6603
M-3222	Palau.79	Porifera	PT	4.96 Pre	01/09/90	B722076	6604
M-3222	Palau.79	Porifera	PT	26.75 Pre	01/09/90	B722077	6605
M-3222	Palau.79	Porifera	PT	3.49 Pre	01/09/90	B722078	6606
M-3223	Palau.79	Porifera	PT	4.05 Pre	01/09/90	B722080	6607
M-3223	Palau.79	Porifera	PT	14.03 Pre	01/09/90	B722081	6608
M-3225	Palau.79	Porifera	PT	1.97 Pre	01/02/90	B722087	6609
M-3225	Palau.79	Porifera	YF	1.05 Pre	01/02/90	"	6609
M-3226	Palau.79	Echinodermata	PT	1.19 Pre	01/02/90	B722089	6610
M-3227	Palau.79	Porifera	PT	11.17 Pre	01/02/90	B722091	6611
M-3228	Palau.79	Porifera	PT	5.28 Pre	01/09/90	B722094	6612
M-3233	Palau.79	Porifera	YF	1.71 Pre	01/09/90	B722109	6613
M-3233	Palau.79	Porifera	YF	3.85 Pre	01/09/90	B722111	6614
M-3243	Palau.79	Coelenterata	PT	1.69 Pre	01/09/90	B722141	6615
M-3250	Palau.79	Porifera	PT	1.23 Pre	01/09/90	B722162	6616
M-3251	Palau.79	Porifera	PT	1.46 Pre	01/09/90	B722165	6617

USAMRIID ACTIVES (Contract)

ASU NUM	AREA	TYPE	VIRUS	DATA SCREEN	DATE RECD	DRUG NUM	AVS NUM
M-3251	Palau.79	Porifera	YF	2.90 Pre	01/09/90	"	6617
M-3252	Palau.79	Porifera	PT	1.63 Pre	01/09/90	B722168	6618
M-3257	Palau.79	Porifera	YF	1.31 Pre	01/09/90	B722181	6619
M-3257	Palau.79	Porifera	PT	2.68 Pre	01/08/90	B722182	6620
M-3257	Palau.79	Porifera	YF	44.17 Pre	01/16/90	"	6620
M-3257	Palau.79	Porifera	PT	7.13 Pre	01/08/90	B722183	6621
M-3257	Palau.79	Porifera	YF	7.48 Pre	01/16/90	"	6621
M-3271	Palau.79	Porifera	PT	13.02 Pre	01/08/90	B722222	6622
M-3271	Palau.79	Porifera	PT	4.98 Pre	01/08/90	B722224	6623
M-3273	Palau.79	Porifera	PT	14.07 Pre	01/08/90	B722228	6624
M-3273	Palau.79	Porifera	PT	4.07 Pre	01/08/90	B722230	6625
M-3276	Palau.79	Porifera	PT	2.41 Pre	01/08/90	B722239	6626
M-3276	Palau.79	Porifera	YF	5.60 Pre	01/08/90	"	6626
M-3277	Palau.79	Porifera	PT	3.12 Pre	01/08/90	B722241	6627
M-3277	Palau.79	Porifera	YF	31.62 Pre	01/08/90	"	6627
M-3279	Palau.79	Porifera	PT	2.26 Pre	01/08/90	B722246	6628
M-3279	Palau.79	Porifera	YF	1.89 Pre	01/08/90	"	6628
M-3279	Palau.79	Porifera	PT	1.47 Pre	01/08/90	B722247	6629
M-3279	Palau.79	Porifera	YF	1.06 Pre	01/08/90	"	6629
M-5523	Wewak.85	Porifera	PT	2.13 Pre	11/14/89	B724373	6630
M-5529	Wewak.85	Porifera	PT	1.59 Pre	09/28/89	B724379	6631
M-5532	Wewak.85	Porifera	PT	3.51 Pre	09/28/89	B724382	6632
M-5534	Wewak.85	Porifera	PT	4.49 Pre	09/28/89	B724384	6633
M-5535	Wewak.85	Porifera	PT	1.86 Pre	09/28/89	B724385	6634
M-5537	Wewak.85	Porifera	PT	1.51 Pre	09/28/89	B724387	6635
M-5537	Wewak.85	Porifera	PT	4.55 Pre	11/14/89	"	6635
M-5544	Wewak.85	Porifera	PT	2.34 Pre	09/28/89	B724394	6636
M-5546	Wewak.85	Porifera	PT	1.16 Pre	09/28/89	B724396	6637
M-5546	Wewak.85	Porifera	PT	1.84 Pre	11/14/89	"	6637
M-5556	Wewak.85	Porifera	PT	1.37 Pre	09/28/89	B724405	6638
M-5557	Wewak.85	Porifera	PT	2.82 Pre	09/28/89	B724406	6639
M-5562	Wewak.85	Porifera	PT	1.21 Pre	09/28/89	B724411	6640
M-5564	Wewak.85	Porifera	PT	3.49 Pre	09/28/89	B724413	6641
M-5566	Wewak.85	Porifera	PT	2.20 Pre	09/28/89	B724415	6642
M-5567	Wewak.85	Porifera	PT	7.17 Pre	09/28/89	B724416	6643
M-5568	Wewak.85	Porifera	PT	5.81 Pre	09/28/89	B724417	6644
M-5569	Wewak.85	Porifera	PT	4.44 Pre	09/28/89	B724418	6645
M-5571	Wewak.85	Porifera	PT	2.53 Pre	09/28/89	B724420	6646
M-5573	Wewak.85	Porifera	PT	3.53 Pre	09/28/89	B724423	6647
M-5583	Wewak.85	Porifera	PT	6.79 Pre	09/28/89	B724433	6648
M-5584	Wewak.85	Porifera	PT	4.58 Pre	09/28/89	B724434	6649
M-5586	Wewak.85	Porifera	PT	1.90 Pre	09/28/89	B724436	6650
M-5589	Wewak.85	Porifera	PT	1.90 Pre	09/18/89	B724439	6651
M-5592	Wewak.85	Porifera	PT	1.91 Pre	09/28/89	B724442	6652
M-5592	Wewak.85	Porifera	YF	1.02 Pre	09/28/89	"	6652
M-5597	Wewak.85	Porifera	PT	28.58 Pre	09/28/89	B724447	6653
M-5603	Wewak.85	Porifera	PT	6.31 Pre	09/28/89	B724453	6654
M-5605	Wewak.85	Porifera	PT	2.75 Pre	09/28/89	B724455	6655
M-5606	Wewak.85	Porifera	PT	10.48 Pre	09/28/89	B724456	6656
M-5607	Wewak.85	Porifera	PT	4.55 Pre	09/28/89	B724457	6657
M-5608	Wewak.85	Porifera	PT	7.84 Pre	09/28/89	B724458	6658

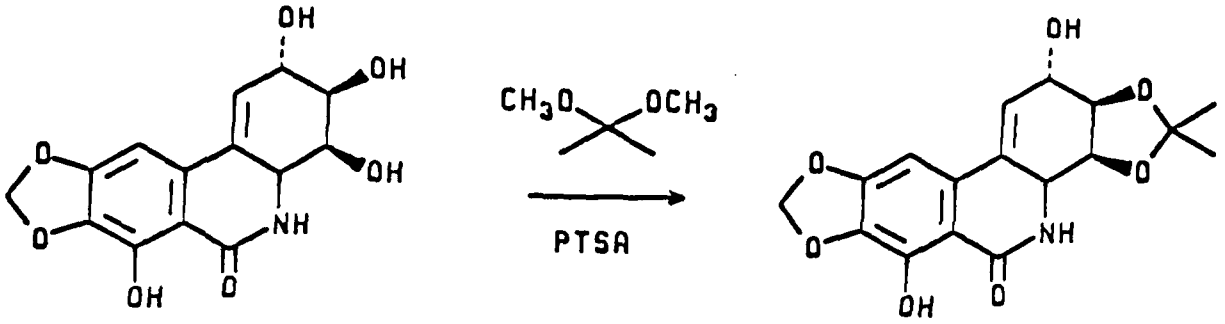
USAMRIID ACTIVES (Contract)

ASU NUM	AREA	TYPE	VIRUS	DATA SCREEN	DATE RECD	DRUG NUM	AVS NUM
M-5616	Wewak.85	Porifera	PT	6.11 Pre	09/28/89	B724466	6659
M-5616	Wewak.85	Porifera	YF	4.77 Pre	09/28/89	"	6659
M-5618	Wewak.85	Porifera	YF	3.33 Pre	09/28/89	B724468	6660
M-5658	Wewak.85	Porifera	PT	3.64 Pre	11/14/89	B724508	6661
M-5659	Wewak.85	Echinodermata	PT	1.28 Pre	11/14/89	B724509	6662
M-5662	Wewak.85	Porifera	PT	5.77 Pre	11/14/89	B724512	6663
M-5667	Wewak.85	Porifera	PT	1.17 Pre	11/14/89	B724517	6664
M-5669	Wewak.85	Porifera	PT	6.58 Pre	11/14/89	B724519	6665
M-5671	Wewak.85	Porifera	PT	11.38 Pre	11/14/89	B724521	6666
M-5675	Wewak.85	Porifera	PT	1.18 Pre	11/14/89	B724525	6667
M-5676	Wewak.85	Porifera	PT	28.78 Pre	11/14/89	B724526	6668
M-5677	Wewak.85	Porifera	PT	7.75 Pre	11/14/89	B724527	6669
M-5680	Wewak.85	Porifera	YF	1.84 Pre	11/14/89	B724530	6670
M-5680	Wewak.85	Porifera	PT	1.26 Pre	11/14/89	"	6670
M-5685	Wewak.85	Porifera	PT	1.44 Pre	11/14/89	B724535	6671
M-5694	Wewak.85	Porifera	PT	1.47 Pre	11/14/89	B724544	6672
M-5736	Wewak.85	Porifera	PT	4.57 Pre	09/28/89	B724586	6677
M-5740	Wewak.85	Coelenterata	PT	2.71 Pre	09/28/89	B724590	6678
M-5742	Wewak.85	Porifera	PT	3.34 Pre	09/28/89	B724592	6679
M-5742	Wewak.85	Porifera	YF	4.94 Pre	09/28/89	"	6679
M-5746	Wewak.85	Porifera	PT	3.44 Pre	09/28/89	B724596	6680
M-5757	Wewak.85	Porifera	PT	3.76 Pre	09/28/89	B724607	6681
M-5760	Wewak.85	Porifera	YF	5.82 Pre	09/28/89	B724610	6682
M-5768	Wewak.85	Porifera	YF	6.69 Pre	09/28/89	B724618	6683
M-5777	Wewak.85	Porifera	PT	2.06 Pre	09/28/89	B724627	6684
M-5783	Wewak.85	Echinodermata	PT	2.32 Pre	09/28/89	B724633	6685
M-5792	Wewak.85	Porifera	PT	1.23 Pre	09/28/89	B724642	6686
M-5794	Wewak.85	Porifera	PT	2.77 Pre	09/28/89	B724644	6687
M-5802	Wewak.85	Echinodermata	PT	3.28 Pre	09/28/89	B724652	6688
M-5804	Wewak.85	Porifera	PT	1.21 Pre	09/28/89	B724654	6689
M-5808	Wewak.85	Porifera	PT	1.52 Pre	09/28/89	B724657	6690
M-5812	Wewak.85	Echinodermata	PT	1.38 Pre	09/28/89	B724661	6691
M-5815	Wewak.85	Porifera	PT	2.36 Pre	09/28/89	B724664	6692
M-5818	Wewak.85	Porifera	PT	1.57 Pre	10/31/89	B724667	6693
M-5821	Wewak.85	Porifera	PT	4.44 Pre	10/31/89	B724670	6250
M-5848	Wewak.85	Porifera	PT	5.38 Pre	10/31/89	B724697	6251
M-5849	Wewak.85	Porifera	PT	11.19 Pre	10/31/89	B724698	6252
M-5852	Wewak.85	Porifera	YF	5.39 Pre	10/31/89	B724701	6253
M-5861	Wewak.85	Porifera	PT	4.77 Pre	10/31/89	B724712	6254
M-5863	Wewak.85	Porifera	PT	2.90 Pre	10/31/89	B724714	6255
M-5865	Wewak.85	Porifera	PT	1.16 Pre	10/31/89	B724716	6256
M-5868	Wewak.85	Porifera	PT	2.97 Pre	10/31/89	B724719	6257
M-5869	Wewak.85	Porifera	PT	1.46 Pre	10/31/89	B724720	6258
M-5871	Wewak.85	Porifera	PT	6.12 Pre	10/31/89	B724722	6259
M-5873	Wewak.85	Porifera	PT	2.56 Pre	10/31/89	B724724	6694
M-5877	Wewak.85	Porifera	PT	3.38 Pre	10/31/89	B724728	6695
M-5878	Wewak.85	Porifera	PT	2.04 Pre	10/31/89	B724729	6696
M-5881	Wewak.85	Porifera	PT	3.06 Pre	10/31/89	B724732	6697
M-5889	Wewak.85	Porifera	PT	20.27 Pre	10/31/89	B724740	6261
M-5912	Wewak.85	Porifera	PT	4.95 Pre	10/31/89	B724762	6262
M-5914	Wewak.85	Porifera	PT	6.41 Pre	10/31/89	B724764	6263

USAMRIID ACTIVES (Contract)

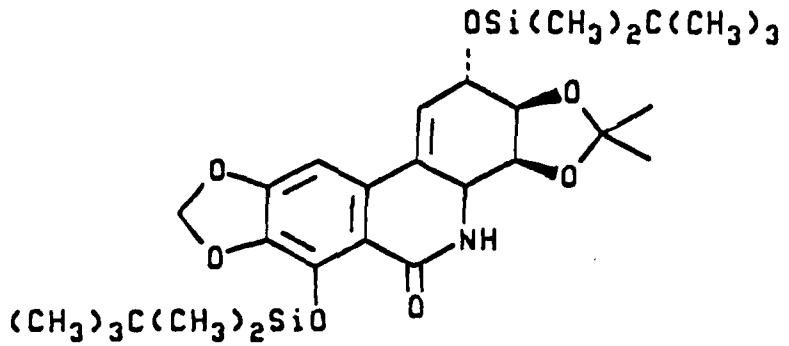
ASU NUM	AREA	TYPE	VIRUS	DATA SCREEN	DATE RECD	DRUG NUM	AVS NUM
M-5919	Wewak.85	Chord./Elasmobra.	PT	1.59 Pre	10/31/89	B724769	6264
M-5922	Wewak.85	Chord./Elasmobra.	YF	1.26 Pre	10/31/89	B724772	6698
M-4894	Maldives.86	Porifera	PT	5.55 Pre	10/31/89	B724781	6265
M-4896	Maldives.86	Porifera	PT	5.69 Pre	10/31/89	B724783	6699
M-4898	Maldives.86	Porifera	PT	2.21 Pre	10/31/89	B724785	6700
M-4910	Maldives.86	Porifera	PT	2.07 Pre	10/31/89	B724797	6266
M-4925	Maldives.86	Coelenterata	YF	1.69 Pre	10/31/89	B724812	6701
M-4933	Maldives.86	Porifera	PT	6.48 Pre	10/31/89	B724820	6268
M-4938	Maldives.86	Porifera	PT	32.91 Pre	10/31/89	B724825	6269
M-4945	Maldives.86	Porifera	PT	1.50 Pre	10/31/89	B724832	6702
M-4957	Maldives.86	Porifera	YF	2.18 Pre	10/31/89	B724844	6270
M-4966	Maldives.86	Porifera	YF	2.09 Pre	10/31/89	B724852	6272
M-4969	Maldives.86	Porifera	PT	3.13 Pre	10/31/89	B724855	6703
M-4974	Maldives.86	Porifera	PT	3.12 Pre	10/31/89	B724860	6274
M-4977	Maldives.86	Porifera	YF	1.81 Pre	10/31/89	B724863	6275
M-4977	Maldives.86	Porifera	PT	2.69 Pre	10/31/89	"	6275
M-4980	Maldives.86	Porifera	PT	1.12 Pre	10/31/89	B724866	6704
M-5001	Maldives.86	Porifera	PT	1.57 Pre	10/31/89	B724885	6705
M-5002	Maldives.86	Porifera	PT	7.84 Pre	10/31/89	B724886	6277
M-5013	Maldives.86	Porifera	YF	8.65 Pre	10/31/89	B724898	6706

SEMI-SYNTHETIC APPROACHES TO PANCRATISTATIN



Narciclasine-3,4-acetonide

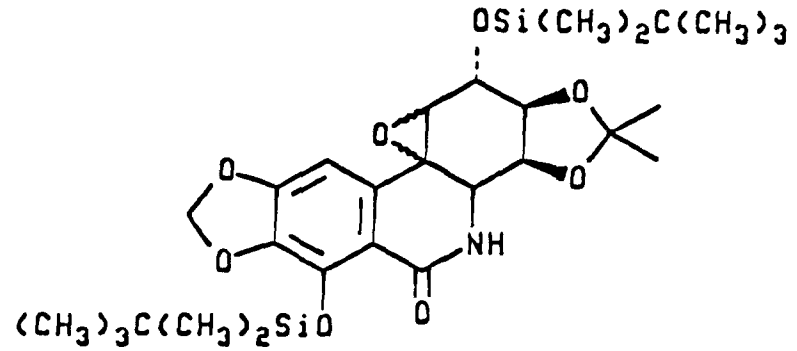
To a solution of narciclasine (1.0 g, 3.25 mmol) in dimethylformamide (5 mL) and dimethoxypropane (5 mL) was added p-toluene sulfonic acid (100 mg). The solution was stirred at room temperature overnight. Acetonide precipitated out of solution. Pyridine (1 mL) and water (50 mL) was added and the mixture was stirred at room temperature for 30 minutes. The precipitate was collected by filtration, washed with water and dried at 64°C over P_2O_5 under high vacuum to give as an amorphous powder, narciclasine-3,4-acetonide (1.05 g, 92.9%), mp. 275-7°, IR (*NaCl*) ν_{max} 3500, 3150, 1637, 1625, 1596, 1464, 1437, 1337, 1201, 1079, 1038, 1019 cm^{-1}, ^{1}HNMR δ ($CDCl_3$) 1.39 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 2.47 (d, J = 4.1 Hz, 1H, OH), 4.10-4.13 (m, 3H, H-3,4,4a), 4.39 (dd, J = 6.7, 4.1 Hz, 1H, H-2), 6.05 (ABq, J = 1.2 Hz, 2H, $-OCH_2O-$), 6.21 (brs, 1H, NH), 6.32 (dd, J = 3, 1.2 Hz, 1H, H-1), 6.70 (s, 1H, H-10), 9.2 (s, 1H, OH).



2,7-Di-[(tert-butyldimethyl)-silyloxy]-narciclasine-3,4-acetonide

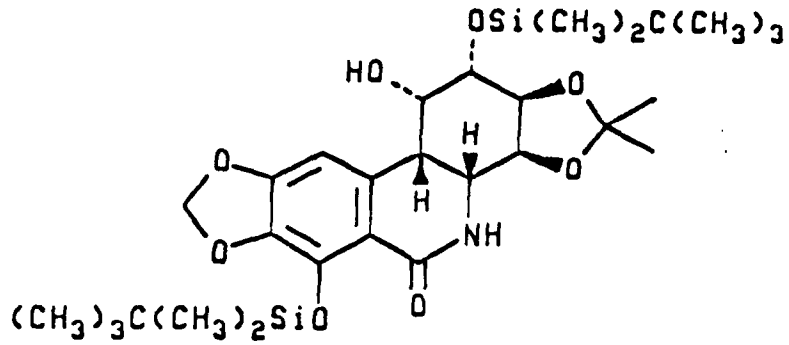
Diisopropylethyl amine (1.8 ml, 10.35 mmol) was added (under argon) to a heated (60°C) solution of narciclasine 3,4-acetonide (800 mg, 2.3 mmol) in dimethylformamide (8 ml) followed by tert-butyldimethylsilyl chloride (1.04 g, 6.9 mmol). The resulting reddish solution was stirred at room temperature overnight and monitored by TLC (hexane: acetone, 4:1). After completion, water (50 ml) was added and the viscous mixture was poured into ether (450 mL). The ethereal solution was washed with 10% aqueous citric acid (50 mL), water (2 x 100 mL), dried and evaporated under reduced pressure to give a gum which was

crystallized from ethanol to afford colorless flakes of disilyloxy derivative (1.2 g, 90.5%), mp. 207-9°C, $[\alpha]_D^{30}$ +61.2°, (c, 2.5, $CHCl_3$), IR (NaCl) ν_{max} 3250, 2952, 2930, 2857, 1676, 1480, 1381, 1362, 112, 1057, 837 cm^{-1}, ^{1}HNMR δ ($CDCl_3$) 0.148, 0.152 (s, 6H, 2xCH_3), 0.219, 0.225 (s, 6H, 2xCH_3), 0.945 (s, 9H, $C(CH_3)_3$), 1.335 (s, 3H, CH_3), 1.467 (s, 3H, CH_3), 3.969-4.019 (m, 2H, 2xCH), 4.065 (dd, J = 7.1, 5.2 Hz, 1H, CH), 4.305 (quint, J = 2.5 Hz, 1H, CH), 5.902 (brs, 1H, NH), 5.967 (d, J = 1.2 Hz, 1H, 1/2OCH_2O), 5.984 (d, J = 1.2 Hz, 1H, 1/2OCH_2O), 6.153 (brt, J = 2.3 Hz, 1H, H-1), 6.799 (s, 1H, H-10).



1,10b-(α)-and-(β)-Epoxy-2,7-di-[(tert-butyldimethyl)silyloxy]-narciclasine-3,4-acetonide

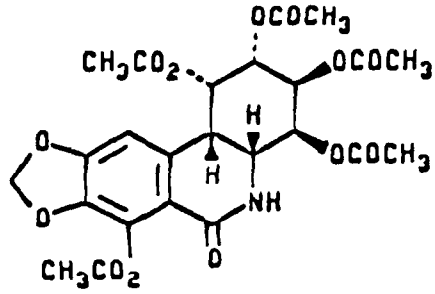
To a stirred solution of 2,7-di-[(tert-butyldimethyl)-silyloxy]-narciclasine-3,4-*acetonide* (240 mg, 0.42 mmol) in CH_2Cl_2 (10 mL) was added 0.2M phosphate (pH 8.0) buffer (10 ml, prepared from Na_2HPO_4 and NaH_2PO_4). The biphasic mixture was cooled to 0°C and m-chloroperbenzoic acid (215 mg, 1.2 mmol) was added and the mixture was stirred 20 min. at 0°C and then 4 hrs. at room temperature. The reaction was carefully monitored (TLC, hexane:acetone, 17:3) and upon completion CH_2Cl_2 (100 mL) was added. The organic phase was separated and washed with 5% aqueous sodium thiosulfate (2 x 25 mL), water (25 mL), 5% aqueous sodium carbonate (3 x 25 mL), water (25 mL), dried (Na_2SO_4), and evaporated under reduced pressure to produce a colorless powder. Chromatography (VLC) on neutral SiO_2 and elution with hexane-acetone (95:5) gave an inseparable mixture (α:β, ^{1}HNMR of hydrogenolyzed product) of epoxides, (205 mg, 83.3% combined yield), mp. 196-9°C, $[\alpha]_D^{30}$ +97.1° (c, 1.05, $CHCl_3$), IR (NaCl) ν_{max} 3350, 2953, 2930, 1680, 1473, 1361, 1344, 1252, 1106, 1063, 1034, 839, 777 cm^{-1}, ^{1}HNMR δ ($CDCl_3$) 0.155 (s, 6H, $SiC(CH_3)_2$), 0.215, 0.242 (s, 3H each, $SiCH_3$), 0.950 (s, 9H, $C(CH_3)_3$), 1.007 (s, 9H, $C(CH_3)_3$), 1.293 (s, 3H, 1/2$C(CH_3)_2$), 1.427 (s, 3H, 1/2$C(CH_3)_2$), 3.811 (s, 1H), 3.869 (d, J = 8.0Hz, 1H), 4.158-4.266 (m, 3H), 5.763 (brs, 1H, NH), 5.972 (d, 1H, J = 1.6 Hz, 1H, 1/2OCH_2O), 6.014 (d, J = 1.6 Hz, 1H, 1/2OCH_2O), 7.294 (s, 1H, H-10), EIMS (m/z) 591 (2%), 576 (10), 534 (100), 518 (4), 476 (10), 430 (10), 344 (4), 316 (6).



(major product)

1α and 1β-Hydroxy-2,7-di-[(tert-butyldimethyl)silyloxy]-10b, 4a-cis and trans-dihydro-narciclasine-3,4-acetonide

To a solution of epoxide mixture (see above) (100 mg, 0.17 mmol) in methanol:ethylacetate (1:3, 20 mL) was added 10% palladium supported on carbon (100 mg). The reaction mixture was evacuated and flushed with hydrogen (5x) and then hydrogenated at ambient temperature and pressure for 1 hr using a hydrogen filled balloon. The catalyst was removed by filtration and the filtrate concentrated to dryness to give a powder (97 mg), purified on PLC (SiO_2, hexane:ethylacetate, 4:1) to give a mixture (1:17, ^{1}HNMR analysis) of 1β, 10bα and 1α, 10bβ alcohols (80 mg, 80%, found to lose the phenolic silyl group in solution), as an amorphous powder from acetone-hexane, mp 116-9°, $[\alpha]_D^{30}$ +7.5° (c, 0.55, $CHCl_3$), IR (NaCl) ν_{max} 3250, 2952, 2929, 1675, 1473, 1382, 1360, 1250, 1220, 1111, 1069, 1042, 839 cm^{-1}, ^{1}HNMR of major product (1α-hydroxy isomer), δ ($CDCl_3$) 0.096, 0.158 (s, 3H each, $Si(CH_3)_2$), 0.231 (s, 6H, $C(CH_3)_3$), 1.403 (s, 3H, CH_3), 1.531 (s, 3H, CH_3), 2.310 (brs, 1H, OH), 2.926 (brs, 1H, H-10b), 3.745 (dd, J = 7.5, 3.3 Hz, 1H, H-2), 3.828 (brs, 1H, H-4a), 4.109 (dd, J = 5.4, 1.5 Hz, 1H, H-4), 4.196 (d, J = 4.0 Hz, 1H, H-1), 4.217 (dd, J = 7.3, 5.3 Hz, 1H, H-3), 5.115 (brs, 1H, NH), 5.930 (d, J = 1.3 Hz, 1H, 1/2OCH_2O), 5.993 (d, J = 1.3 Hz, 1H, 1/2OCH_2O), 6.415 (s, 1H, H-10).

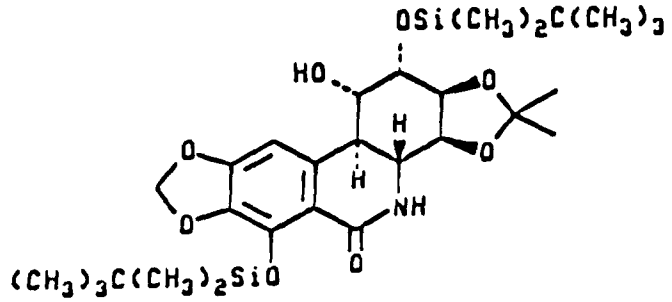


(major product)

1α,2α,3β,4β,7-pentacetyl-10b,4a-cis-dihydro-narciclasine (1α,10bβ-isopancratistatin pentaacetate) and Pancratistatin pentaacetate

To a cooled (0°C) solution of the mixture of silylether (see above, 15 mg, 0.025 mmol) in methanol (2 mL) and water (0.5 ml) was added acetic acid (0.5 ml) and trifluroacetic acid (0.5 ml). The solution was stirred at 0°C for

2 hrs and then stored in a refrigerator overnight. Solvents were removed under reduced pressure and the resulting product was dried under high vacuum over phosphorous pentoxide for 4 hrs. The product was then acetylated using pyridine (0.5 mL) and acetic anhydride (0.5 mL) at room temperature overnight followed by heating at 60°C for 1 hr. The reaction mixture was quenched with methanol and the volatile materials were evaporated through azeotropic distillation with methanol and cyclohexane. Product was found to be a (1:9) mixture of pancratistatin pentaacetate (detected only by NMR spectrum of the mixture) and 1α, 10bβ-isopancratistatin pentaacetate. The products were separated on a column of silica gel by elution with CH_2Cl_2:CH_3OH, 99:1 to give 9.0 mg of an amorphous powder from CH_2Cl_2-hexane of 1α,2α,3β,4β,7-pentaacetyl-10b,4a-cis-dihydro-narciclasine, mp.165-9°, $[\alpha]_D^{30}$ +135 (c, 0.2, $CHCl_3$), IR (NaCl) ν_{max} 3341, 1778, 1751, 1677, 1481, 1371, 1248, 1226, 1192, 1084, 1035 cm^{-1}, ^{1}HNMR δ ($CDCl_3$) assignment based on ^{1}H, ^{1}H-COSY spectra, 1.893, 1.979, 2.027, 2.166, 2.351 (each s, 3H each, 5 x $OCOCH_3$), 3.305 (t, J = 3.8 Hz, 1H, H-10b), 3.933 (t, J = 2.5 Hz, 1H, H-4a), 5.405 (dd, J = 10.7, 3.2 Hz, 1H, H-2), 5.460 (brs, 1H, NH), 5.470 (dd, J = 10.7, 2.3 Hz, 1H, H-3), 5.488 (brs, 1H, H-4), 5.532 (t, J = 3.4 Hz, 1H, H-1), 6.068 (d, J = 1.2 Hz, 1H, 1/2OCH_2O), 6.085 (d, J = 1.2 Hz, 1H, 1/2OCH_2O), 6.626 (s, 1H, H-10), the chemical shift for NH shifted downfield at δ 5.590 in dilute solutions (ca. 1.5 mg/0.5 mL). *Cis* relationship of the protons at H-4a, H-10b and H-1 established by NOE measurement. Thus strong NOE's were observed between H-10b, H-1, H-2, H-10, H-4a, and H-4a also gave NOE enhancement to NH). The NOE's also establishes proof for the chair conformation of ring C.

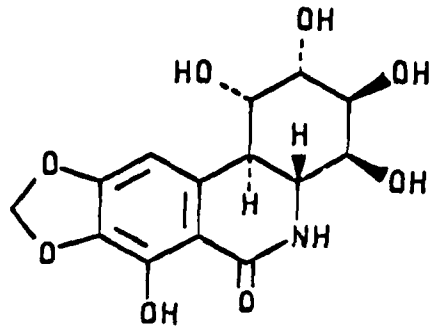


(major product)

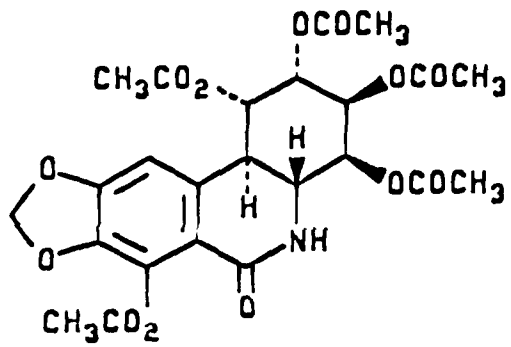
1α-Isopancratistatin

Palladium/carbon (10%, 80 mg) was added to the epoxide mixture described above (80 mg, 0.14 mmol) in anhydrous THF (45 mL) and the hydrogenolysis was performed as described in the previous experiment for 8 hrs. The filtrate obtained after removal of the catalyst was concentrated to dryness to give a 2:1 mixture of trans:cis dihydro product, by ^{1}HNMR analysis. The products were separated on PLC (hexane-acetone (17:3) to give *trans* dihydro product (42 mg, 52.3%) and *cis* dihydro product (20 mg, 24.8%), identical (^{1}HNMR and TLC) with the cis product obtained in the previous hydrogenolysis reaction. Slightly impure *trans* dihydro product was obtained as an amorphous powder from acetone-hexane; ^{1}HNMR δ ($CDCl_3$) of major product: 0.104, 0.138, 0.190, 0.199 (each s, 3H each, 2 x $Si(CH_3)_2$), 0.902, 0.971 (each s, 9H each, 2 x $C(CH_3)_3$), 1.312, 1.433 (each s, 3H each, $C(CH_3)_2$), 2.814 (dd, J = 14.3, 7.7 Hz, 1H, H-10b), 3.085 (d, J = 3.7 Hz, 1H, OH), 3.457 (dd, J = 14.1, 8.0 Hz, 1H, H-4a), 3.866 (dd, J = 7.2, 5.0 Hz, 1H, H-2), 4.055 (m, 1H, H-1), 4.207 (t, J = 8.5

Hz, 2H, H-3,4), 5.696 (*s*, 1H, NH), 5.910 (*d*, *J* = 1.2 Hz, 1H, 1/2OCH_2O), 5.950 (d, *J* = 1.2 Hz, 1H, 1/2OCH_2O), 6.998 (*s*, 1H, H-10).



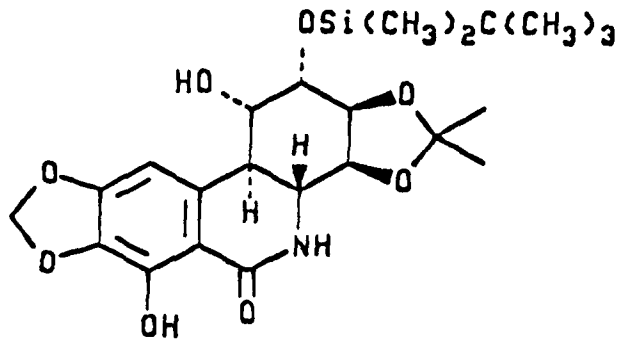
To a ccoled (0°C) solution of <u>trans</u> product (25 mg 0.042 mmol) in THF:CH_3OH:H_2O (1.5:2:1, 4.5 mL) was added acetic acid (0.5 mL) and trifluoroacetic acid (1.0 mL) and stirred at the same temperature for 1 hr. After storing overnight in the refrigerator, the reaction was not complete and required heating to 40°C for 8 hrs. Solvents were removed under reduced pressure and the product was purified by flash chromatography on silica gel. The product, 1α-isopancratistatin (11.1 mg, 81%), eluted with a 9:1 mixture of CH_2Cl_2:CH_3OH and was obtained as an amorphous powder, mp. 325-7, IR (KBr) ν_{max} 3500-3300, 1679, 1470, 1337, 1285, 1210, 1141, 1089, 1064, 802, 725 cm^{-1}, ^{1}HNMR δ (DMSO-d_6+D_2O) 2.80 (dd, 10.9, 10.9 Hz, 1H, H-10b), 3.31 (*dd*, *J* = 13.2, 10.5 Hz, 1H, H-4a), 3.76 (m, 2H), 3.81 (t, *J* = 3.6 Hz, 1H), 3.84 (brs, 1H), 5.97, 6.00 (only two AB lines visible, 2H, OCH_2O), 7.27 (*s*, 1H, H-10).



1α,2α,3β,4β,7-Pentaacetyl isopancratistatin

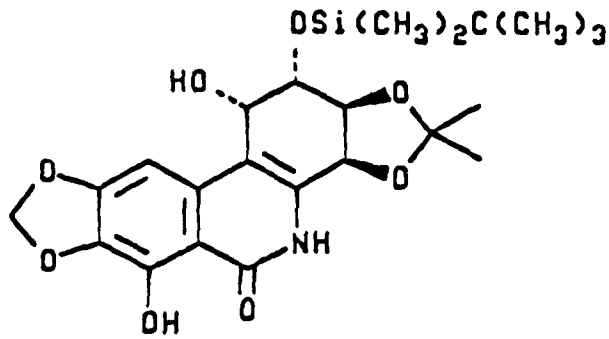
1α-Isopancratisatin (2.7 mg) was treated with acetic anhydride (0.2 mL) in pyridine (0.2 ml) at 50°C for 2 hrs. The mixture, quenched with methanol, was reduced to dryness under a nitrogen stream. Product was chromatographed on a column of silica gel and eluted with CH_2Cl_2:CH_3OH (49:1) to give an amorphous powder of 1α-isopancratistatin pentaacetate (3.4 mg, 76.5%), mp. 146-8, IR (NaCl) ν_{max} 3350, 2930, 2850, 1755, 1676, 1482, 1370, 1248, 1226, 1176, 1084, 1059, 1033 cm^{-1}, ^{1}HNMR δ ($CDCl_3$) 2.06 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 2.18 (s, 3H, $COCH_3$), 2.36 (s, 3H, $COCH_3$), 3.44 (t, *J* = 11.9 Hz, 1H, H-10b), 3.84 (t, *J* = 11.0 Hz, 1H, H-4a), 5.25 (dd, *J* = 10.8,

3.0 Hz, 1H, H-4), 5.42 (dd, J = 11.4, 3.3 Hz, 1H, H-1), 5.44 (t, J = 3.3 Hz, 1H, H-1), 5.53 (t, J = 3.8 Hz, 1H, H-2), 6.06 (d, J = 1.2 Hz, 1H, 1/2OCH_2O), 6.07 (d, J = 1.2 Hz, 1H, 1/2OCH_2O), 6.54 (s, 1H, H-10), spectrum was assigned on the basis of 2D-COSY, and the stereochemistry by NOEDS data.



1α-Hydroxy-2-[(tert-butyldimethyl)silyloxy]-10b, 4a-cis and trans-isopancratistatin-3,4-acetonide and 1α-hydroxy-2-[(tert-butyldimethyl)silyloxy]-Δ(10b,4a)-isopancratistatin-3,4-acetonide

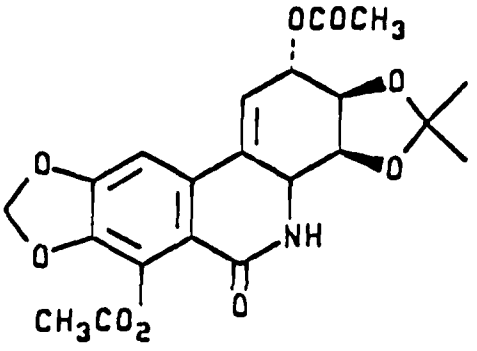
The silyloxy epoxide mixture (150 mg) was dissolved in THF (10 mL) and hydrogenolyzed using hydrogen-10% Pd/C (50 mg) as described. Chromatography on a silica gel column and elution with hexane-acetone (7:3) gave 7-desilylated products (interestingly desilation was occurring during the hydrogenolysis reaction as crystallized epoxide was free of benzoic acid by NMR) trans:cis:Δ(10b,4a) in the ratio of (5:3:5). The 10b,4a trans product (50 mg, 38%), crystallized from methanol as shining flakes, mp. 274-5; IR (NaCl) ν_{max} 3530, 3360, 2952, 2939, 1678, 1466, 1373, 1361, 1345, 1260, 1230, 1085, 1071 cm^{-1}; ^1H NMR δ ($CDCl_3$) 0.15 (s, 3H, $SiCH_3$), 0.18 (s, 3H, $SiCH_3$), 0.94 (s, 9H, $SiC(CH_3)$), 1.37 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 2.90 (dd, J = 14.3, 6.9 Hz, 1H, H-10b), 3.13 (d, J = 2.7 Hz, 1H, OH), 3.57 (dd, J = 14.5, 7.9 Hz, 1H, H-4a), 3.92 (dd, J = 6.8, 5.2 Hz, 1H, H-2), 4.13 (ddd, J = 7.4, 4.6, 2.2 Hz, 1H, H-1), 4.25 (t, J = 6.7 Hz, 1H, H-4), 4.29 (t, J = 6.7 Hz, 1H, H-3), 6.03 (ABq, J = 3.0 Hz, 2H, OCH_2O), 6.04 (brs, 1H, NH), 6.93 (s, 1H, H-10), 12.48 (s, 1H, ArOH), (assignment was made on the basis of a 2D-COSY analysis and stereochemical assignment was accomplished by NOEDS measurement).



Continued elution of the column with the same solvent gave a mixture of cis and 10b,4a (Δ) product. The cis product could not be separated

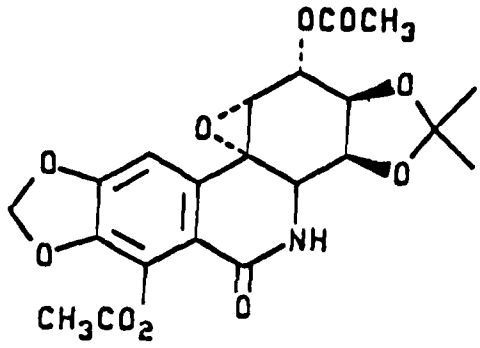
but crystallization of the mixture from methanol yielded pure 10b, 4a (Δ) olefinic product (55 mg, 41.4%), recrystallized from methanol as flakes, mp. 266-8; IR (NaCl) ν_{max} 3535 (brs), 2989, 2959, 2931, 2897, 2857, 1677, 1625, 1485, 1422, 1373, 1253, 1215, 1117, 1086, 1036 cm^{-1}; ^1H NMR δ ($CDCl_3$) 0.17 (s, 3H, $SiCH_3$), 0.21 (s, 3H, $SiCH_3$), 0.97 (s, 9H, $SiC(CH_3)$, 1.49 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 2.90 (s, 1H, OH), 3.86 (dd, J = 7.8, 3.5 Hz, 1H, H-2), 4.50 (t, J = 7.5 Hz, 1H, H-3), 4.76 (d, J = 3.4 Hz, 1H, H-1), 5.11 (d, J = 7.0 Hz, 1H, H-4), 6.10 (d, J = 1.6 Hz, 1H, 1/2OCH_2O), 6.11 (d, J = 1.6 Hz, 1H, 1/2OCH_2O), 6.83 (s, 1H, H-10), 9.85 (s, 1H, NH), 12.65 (s, 1H, OH), Assignment is based on 2D-COSY spectrum and stereochemistry was determined by NOEDS measurement.

Hydrogenolysis of the silyloxy epoxide mixture on a scale better than the one reported here in different solvents (ethyl acetate, mixture of ethyl acetate and methanol) produced similar products. Hydrogenolysis in methanol mostly produced the Δ (10b, 4a) product.



2,7-Diacetoxy-narciclasine-3,4-acetonide

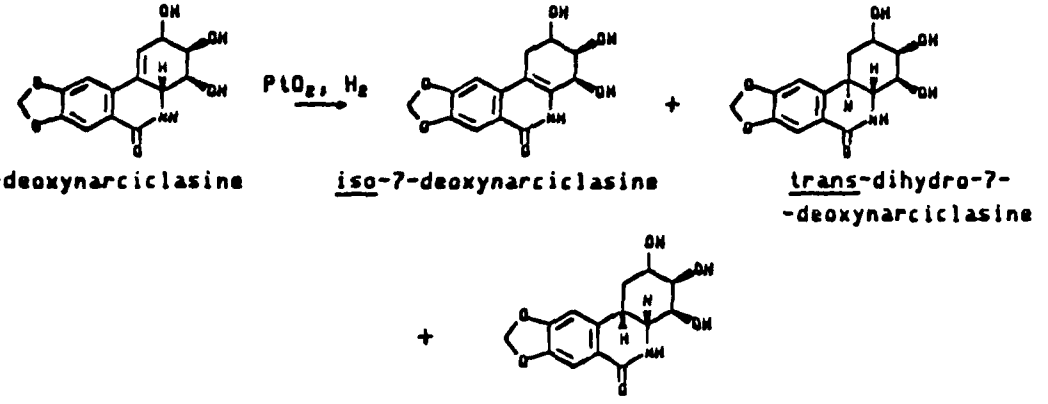
Acetonide was prepared from narciclasine (1 g) as described before and all the solvents were evaporated under reduced pressure to give a crude product which was acetylated with acetic anhydride (3 mL) - pyridine (3 mL) at 60°C for 6 hrs. Solvents were evaporated under reduced pressure after addition of methanol and then chromatographed on a silica gel column and eluted with hexane-ethyl acetate-methylene chloride (3:1:2) to give pure diacetate (1.2 g, 85.4%) as an amorphous powder from acetone-hexane, mp. 130-33 °C; IR (NaCl) ν_{max} 3350, 1775, 1745, 1671, 1482, 1373, 1233, 1210, 1177, 1081, 1031 cm^{-1}; ^1H NMR δ ($CDCl_3$) 1.39 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 2.21 (s, 3H, $COCH_3$), 2.39 (s, 3H, $COCH_3$), 4.12 (dd, J = 7.8, 7.8 Hz, 1H, H-3), 4.16 (brs, 1H, H-4a), 4.31 (dd, J = 7.5, 5.8 Hz, 1H, H-4), 5.39 (dd, J = 4.9, 2.3 Hz, 1H, H-2), 6.02 (brs, 1H, NH), 6.09 (brs, 2H, OCH_2O), 6.12 (t, J = 3.2 Hz, 1H, H-1), 6.98 (s, 1H, H-10).



1,10b-(α)-Epoxy-2,7-diacetoxy-narciclasine-3,4-acetonide

To a solution of narciclasine acetonide diacetate (1.0 g, 2.32 mmol) in CH_2Cl_2 (60 mL) was added 0.2 M soadium phosphate buffer (pH 8, 60 mL) followed by m-chloroperbenzoic acid (1.4 g, 3.5 molar equivalent). The reaction mixture was stirred at room temperature overnight and then CH_2Cl_2 (500 mL) was added and the organic layer was separated, washed with 5% solution of sodium thiosulfate (3 x 200 mL), 5% solution of sodium carbonate (3 x 200 mL), water (2 x 100 mL) , dried (Na_2SO_4) and evaporated to give almost pure epoxide as a powder, crystallized from acetone-hexane (700 mg, 67.5%) as amorphous granules, mp. 231-232 °C; IR (NaCl) ν_{max} 3370, 1792, 1749, 1682, 1500, 1365, 1345, 1209, 1175, 1083, 1032 cm^{-1}; ^1H NMR δ ($CDCl_3$) 1.31 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 2.20 (s, 3H, $COCH_3$), 2.36 (s, 3H, $COCH_3$), 4.00 (d, J = 6 Hz, 1H, H-4a), 4.03 (s, 1H, H-1), 4.27 (apparent t, J = 8.1 Hz, H-1, H-3), 4.38 (dd, J = 7.9, 6.3 Hz, 1H, H-4), 5.33 (d, J = 6.1 Hz, 1H, H-2), 5.82 (brs, 1H, NH), 6.07 (ABq, J = 1.2 Hz, OCH_2O), 6.43 (s, 1H, H-10).

SYNTHETIC TRANSFORMATION OF 7-DEOXYNARCICLASINE



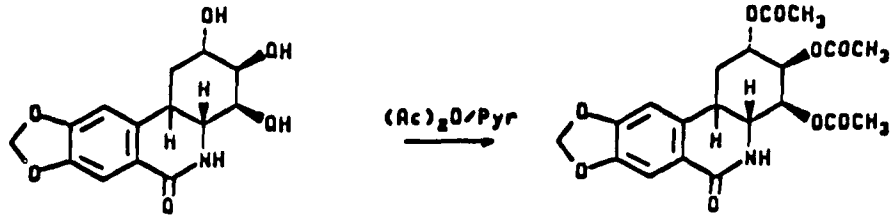
7-deoxynarciclasine

iso-7-deoxynarciclasine

trans-dihydro-7-deoxynarciclasine

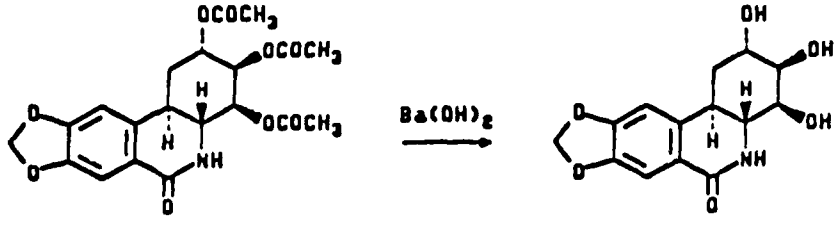
cis-dihydro-7-deoxynarciclasine

Hydrogenation of 7-deoxynarciclasine. A solution of 7-deoxynarciclasine (1.02 g, 3.4 mmol) in methanol-ethanol (400 ml, 1:1) was degassed with nitrogen, platinum oxide (57 mg) was carefully added and the resulting mixture was hydrogenated at ambient temperature and pressure for 24 hrs. The reaction mixture was filtered through Celite and concentrated in vacuo to afford crude iso-7-deoxynarciclasine (150 mg, dark brown solid) which crystallized from pyridine-hexane as a powder (100 mg, 9.8% yield). Identity with earlier sample of the compound was confirmed by mp and nmr. The mother liquor contained cis and trans-dihydro-7-deoxynarciclasine.

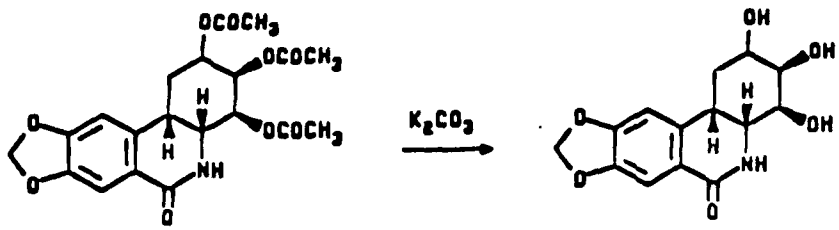


cis- and trans-isomers cis- and trans-isomers

cis and trans-dihydronarciclasine triacetate. The crystallization residue from iso-7-deoxynarciclasine (see above) was concentrated to dryness and treated with acetic anhydride (7 ml) and pyridine (10 ml) at 60° for 6 hrs. Methanol was added and the resulting solution was concentrated to dryness. The mixture was flash chromatographed over silica gel using CH_2Cl_2:MeOH (99.4-0.6) twice to furnish trans-dihydro-7-deoxynarciclasine (112 mg, 7.6% yield) and cis-dihydro-7-deoxynarciclasine (752 mg, 51.2% yield). Identity with earlier sample of the compound was confirmed by mp and nmr.



Trans-dihydro-7-deoxynarciclasine. To a solution of the triacetate (112 mg) in methanol (20 ml) was added a saturated solution of barium hydroxide (6 ml). After heating for 15 min. at 100°C, the mixture was cooled, saturated with solid CO_2, stirred at room temperature overnight and filtered. The filtrate was evaporated to dryness and the product was crystallized from methanol to give trans-dihydro-7-deoxynarciclasine (51 mg, 65% yield). Identity with earlier sample of the compound was confirmed by mp and nmr.

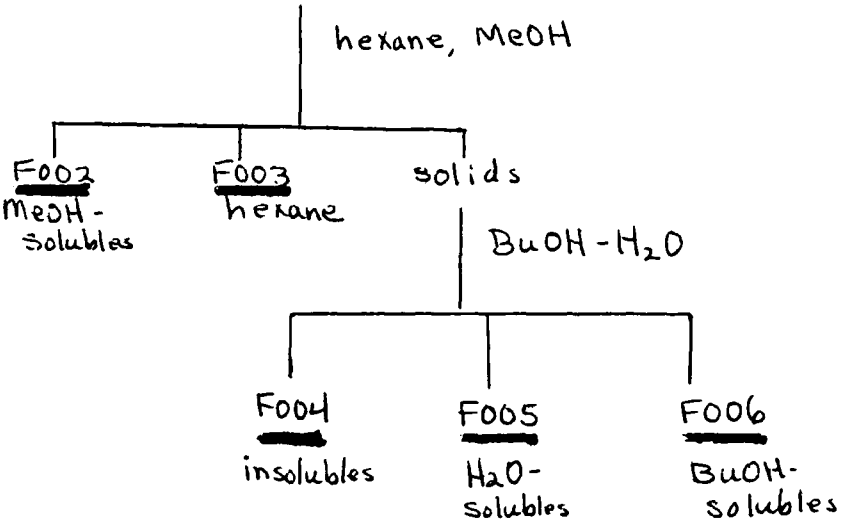


cis-dihydro-7-deoxynarciclasine. To a solution of the triacetate (750 mg) in methanol (80 ml) was added potassium carbonate (300 mg). The mixture was stirred at room temperature for 2 hrs, then filtered through a column of Sephadex LH-20. Elution with CH_2Cl_2:MeOH (3:2) removed the product from the column. Crystallization from acetone-MeOH afforded cis-dihydro-7-deoxynarciclasine as crystals (419 mg, 80% yield). Identity with earlier sample of the compound was confirmed by mp and nmr.

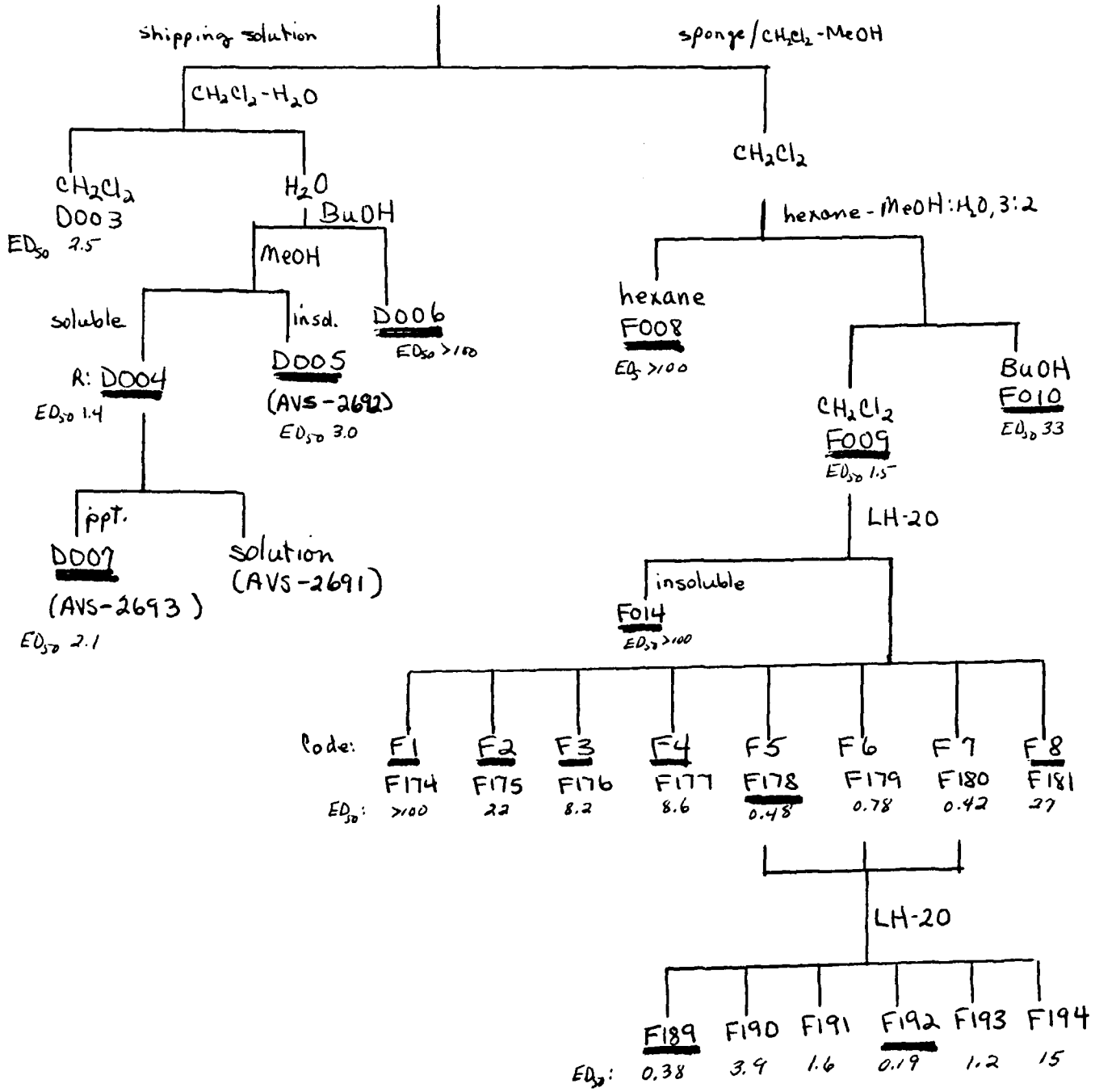
Philippines Sponge

B721160, Haliclona sp. (Porifera)

aqueous extract
(AVS-1902)



Papua New Guinea Sponge B723344 (Porifera)



CONCLUSIONS

The discovery that pancratistatin will cure USAMRIID's in vivo Japanese Encephalitis has opened the way to a new generation of antiviral drugs. Doubtlessly, current efforts at uncovering new naturally occurring antiviral drugs will lead to analogous excellent progress.

REFERENCES

Summary of Manuscripts Already Submitted

George R. Pettit, Atsushi Numata, Chika Takahashi, Tamie Miyamoto, Dennis L. Doubek, Ryoko Fujiki, and Delbert L. Herald, "Isolation and Structure of Cytostatic Steroidal Saponins from the African Medicinal Plant, Balanites aegyptiaca," Chem. Pharm. Bull. (Japan)

George R. Pettit, Atsushi Numata, Tsuruko Takemura, Richard H. Ode, A. S. Narula, Jean M. Schmidt, Gordon M. Cragg, and Charles P. Pase, "Antineoplastic Agents. 107. Isolation of Acteoside and Isoacteoside from Castilleja linariaefolia (Scrophulariaceae)," J. Nat. Prod.

George R. Pettit, Cherry L. Herald, Rajesh Gupta, John E. Leet, Daniel E. Schaufelberger, Robert B. Bates, Paul J. Clewlow, Dennis L. Doubek, Kirk P. Manfredi, Klaus Rutzler, Jean M. Schmidt, Franklin B. Ward, Michael Bruck, and Fernando Camou, "Antineoplastic Agents. 168. Isolation and Structure of Axinohydantoin," Can. J. Chem.

George R. Pettit, Gordon M. Cragg, Sheo Bux Singh, James A. Duke, and Dennis L. Doubek, "Antineoplastic Agents. 162. Zephyranthes candida," J. Nat. Prod.